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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	S. Charbit et al.
Serial No.	:	09/768816
Filed	:	1/23/01
For	:	TREATMENT OF PATHOLOGICAL CONDITIONS CHARACTERIZED BY AN INCREASED IL-1 LEVEL
Attorney Docket No.	:	H7708-0002
Examiner	:	Mojdeh Bahar
Art Unit	:	1617

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

Declaration Of Diego Provvedini Under 37 C.F.R. § 1.132

I, Diego Provvedini, M.D., declare and say that:

My residence address is: 55 Rue Jacques Kellner, 78380 Bougival, France.

I am a Physician trained in Italy and the United States in basic and clinical research focusing on the musculoskeletal system, and currently employed in the pharmaceutical industry in France. A copy of my Curriculum Vitae is attached hereto as Exh. A.

I understand the treatment of pathological conditions characterized by an increased IL-1 and/or TNF-α level through the administration of diacerein and/or rhein, as disclosed and claimed in the above-identified patent application.

I have reviewed the above-identified patent application disclosure and the prior art references cited by the Examiner in the Office Action, namely the Martel-Pelletier et al. reference and the Marcolongo et al. reference.

A person of ordinary skill in this particular field would not have been motivated to combine the *in vitro* results of Martel-Pelletier et al. with Marcolongo's symptomatic treatment regimen and the statements on page 1 of the patent application, and have a reasonable expectation that an underlying pathological cause of the claimed group of diseases would be successfully treated. As a general matter, it is not possible to link *in vitro* observations of the effectiveness of a drug with other results independently observed *in vivo* in another study. The clinician of ordinary skill very often observes that several mechanisms are involved in the expression of a pathologic condition in a patient. Thus, even in the *in vitro* systems that most closely model *in vivo* conditions, these same mechanisms are not necessarily present and/or involved, due to many possible reasons such as a lack of critical components and elements, or the insufficiency of the observation times. Furthermore, in *in vivo* testing the deficit of one mechanism can be compensated by others, thereby maintaining an overall balanced situation. These homeostatic and compensatory mechanisms may further explain the absence of a correlation between the *in vitro* versus the *in vivo* effectiveness of a drug that is very often observed during pharmacological development.

The matrix metalloprotease (MMP) inhibitors represent a good example of the existence of the "disconnect" between *in vitro* versus *in vivo* data. Considerable efforts have been made to produce MMP inhibitors as therapeutic agents for chondro-destructive pathologies. Thus, several MMP inhibitors have entered clinical trials, but have never reached the market. The most recent example is Roche's *Trocade*, a selective inhibitor of collagenase (MMP-1 and -

3, and, at least in part, -13 and -14) which had been extensively tested in pre-clinical settings, but whose development was terminated after a phase III study in rheumatoid arthritis (RA) showed no effect whatsoever. The explanation for the failure of *Trocade*, a powerful MMP inhibitor *in vitro*, is not known. This is even more intriguing in view of the fact that an antagonist of MMPs, the tissue inhibitor of metalloprotease (TIMP), can completely block the destruction of the collagen induced *in vitro* by the MMPs.

A similar example of the "disconnect" between *in vitro* versus *in vivo* data is represented by the statins, powerful cholesterol-lowering agents. Considerable enthusiasm had been generated by the observation of their beneficial effects on bone metabolism, which induced to propose them as a potential treatment for bone-losing pathologies, and osteoporosis in particular. Their promising *in vitro* effect on the enzyme HMG-CoA reductase and the maturation of the bone-resorbing osteoclasts has not been confirmed *in vivo* during several large, controlled clinical trials. Therefore, at the present time statins are no longer considered as a viable alternative treatment for osteoporosis.

Another very clear and fitting example of the existence of a disconnect between *in vitro* versus *in vivo* data is represented by the involvement of the cytokine IL-1 in the mechanisms of the immune response. Indeed, a large body of *in vitro* experimental evidence has demonstrated beyond doubt that IL-1 plays a pivotal role in the activation of the immune cells during their response to an immune stimulus. Thus, it is logical to believe that the inhibition of the production, levels or activity of this cytokine would have some effect on an immune response *in vivo*. Nevertheless, in a total population of more than 3,000 patients receiving diacerein during controlled clinical trials no inhibitory effect on immunity due to the inhibition of IL-1 was ever demonstrated.

It needs to be pointed out that the IL-1 system is very complex (*Arend W P and Guthridge C J: Biological role of interleukin 1 receptor antagonist isoforms. Ann Rheum Dis 2000; 59 (suppl 1): 160 –4*, attached as Exh. B): there are at least 2 ligands (IL-1α and IL-1β) and at least 2 receptors (IL-1RI and IL-1RII). In addition, the main IL-1 antagonists are represented by one secreted form (called sIL-1Ra), produced by monocytes and macrophages, and three different intracellular isoforms, whose biological role is still unclear, namely: 1) icIL-1Ra1 (a major protein in keratinocytes and other epithelial cells); 2) icIL-1Ra2 (in neutrophils, fibroblasts, keratinocytes and myelomonocytic cells); and 3) icIL-1Ra3 (a major protein in hepatocytes and neutrophils, and in smaller amounts in monocytes, macrophages and keratinocytes). The effect of a drug or treatment on IL-1 might therefore depend upon several different actions on various elements of this cytokine's homeostatic system, such as the interleukin-converting enzyme (ICE), the receptor(s), the antagonist(s), or directly on the IL-1 molecule itself.

Thus, it is clear that the field of art in the above-identified application is particularly complex and highly unpredictable so that a person of ordinary skill could not reasonably conclude that the same effects observed *in vitro* would be obtained when diacerein is administered *in vivo*.

I am also familiar with the data underlying, and the work surrounding, the Martel-Pelletier et al. reference. In my opinion, as stated above, the highly complex nature of this field would preclude one of ordinary skill in the art from making any reasonable predictions that diacerein or rhein would prevent the progression of osteoarthritis in humans when administered to humans based only on the reported *in vitro* results of the paper. The authors themselves apparently understood that they needed to see the results of the human trials before assessing the

clinical relevance of the *in vitro* results in their paper, and therefore did not have an expectation that the *in vitro* model would necessarily correlate with a positive clinical outcome. Indeed, for this purpose, they stated in their paper: "Diacerein is currently under investigation *in vivo* in patients with hip and knee osteoarthritis to explore its potential structure modifying effect. The latter should yield useful information regarding the clinical relevance of this *in vitro* study." Of course they may have been hopeful that the *in vitro* results would carry over to the *in vivo* results, but they expressed no reasonable expectation that their *in vitro* results would do so.

Based on my reading of the above-identified patent application, one of ordinary skill in the art would understand that pulmonary fibrosis would be included within the group of inflammatory and autoimmune diseases described in the application. First, one of ordinary skill in the art would have known that pulmonary fibrosis is an inflammatory disease of the lungs characterized by increased IL-1 and TNF-α levels. Second, the application contains multiple references to inflammatory diseases characterized by increased IL-1 and TNF-α levels. For example, p. 1, lns. 10-11 ("The invention specifically resides in a method for treatment of pathological conditions characterized by an increased IL-1 and/or TNF-α level..."), p. 6, lns. 14-17 ("An objective of the invention was to provide a method of treatment [for] patients suffering from the inflammatory and autoimmune diseases, in which inflammatory cytokines, such as interleukin-1 (IL-1) and tissue necrosis factor α (TNF-α) are present to an increased degree..."), claim 1 as originally filed ("Method of treating pathological conditions characterized by an increased IL-1 and/or TNF-α level...") and claim 11 as originally filed ("Method of treating inflammatory and autoimmune conditions characterized by an increased IL-1 and/or TNF-α level..."). Furthermore, the specification throughout states that "the pathological conditions contemplated herein ... broadly encompass the inflammatory and autoimmune diseases" (p. 1,

lns. 18-19), "the use of diacerein and rhein in the treatment of inflammatory and autoimmune diseases" (p. 4, ln. 7), "method of treatment including the administration of diacerein or rhein to patients suffering from the inflammatory and autoimmune diseases" (p. 6, ln. 14-16), Claim 2, etc. Attention is also called to p. 5, lns. 17-18, wherein it is noted that "IL-1 and TNF... contribute to the fibrosis and tissue degeneration of the chronic proliferative phase of inflammation" (emphasis added). Based on these disclosures in the application, I conclude that one of ordinary skill in the art would recognize from the disclosure that the application contains an adequate description of the general group of diseases characterized by increased IL-1 and TNF-α levels such as to lead one of ordinary skill in the art to understand that pulmonary fibrosis is included within this group.

I declare further that all statements made herein of my own knowledge are true, and that all statements made on information are believed to be true; and, further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully Submitted,

Diego Provvedini

Dated: September 17, 2002